A COMPARATIVE ANTIFUNGAL STUDY OF CORIANDER OIL AND ITS NANO EMULSION

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Abstract: Nanoemulsions have potential advantages over conventional emulsions due to their unique physicochemical properties such as very small droplet size, optical transparency and long term physical stability. In this study, nanoemulsion was formulated from essential oil of coriander and non-ionic surfactant Tween 80 by ultrasonic emulsification method. Transparent nanoemulsion with mean droplet diameter of 146.9 ± 1.9 nm was obtained at 8% oil concentration in nanoemulsion and was found to be stable. Physicochemical characterization (hydrodynamic diameter, thermodynamic stability and zeta potential) was carried out. A comparison of activity of coriander oil and its nanoemulsion against *Candida albicans, Candida tropicalis* and *Candida parapsilosis* was studied. The nanoemulsion demonstrated time and dose dependent fungicidal activity. The results suggested that the formulated nanoemulsion was more potent than coriander oil alone. Nanoemulsion was found to be effective at as low as 4% concentration, signifying much lesser coriander oil concentration (0.32%) with respect to all the three *Candida* spp.

Keywords: Coriander oil, Nanoemulsion, Ultrasonic emulsification, Tween 80, *C. albicans, C. tropicalis, C. parapsilosis*, zeta potential

Introduction: The overgrowth of *Candida spp.* in the vagina is termed as vaginal candidiasis or more commonly, thrush [1]. The normal vaginal flora is dominated by *Lactobacillus* spp. which acidifies the environment and prevents pathogenesis [2].*Candida* spp. is also found to infect medical devices, such as venous and urinary catheters, by adhering to the surface and forming a community of drug-resistant cells surrounded by a matrix. The most clinically important phenotype of Candida biofilm cells is their remarkable resistance to antifungal drugs. Cells in this environment can survive up to 1,000-fold higher concentrations of antifungals than nonbiofilm, planktonic cells [3]-[4].

Novel drug targets and the development of new antifungal agents for the treatment of these recalcitrant infections are therefore of interest. Essential oils have been reported to be active against *Candida* spp., suggesting that they may be useful in the topical treatment of superficial candidal infections [5]. Coriander oil has been shown to have an effective antimicrobial activity against *Candida* spp., which could be useful in designing new formulations for candidiasis treatment [6].

There is also tremendous increasing interest in the area of plant oil nanoemulsions due to its bioavailability and biocompatibility leading to their applications in material science, medicine, pharmacology and agriculture. Nanoemulsions are obtained when the size of an emulsion globule reaches approximately 20-500 nm. [7]-[8]. Nanoemulsions are primarily produced either by high-energy emulsification (e.g., high-pressure homogenisation) or by low-energy emulsification (using physicochemical properties of the components).

The objective of the present study was to formulate plant essential oil based nanoemulsion by ultrasonic emulsification method and to compare the antifungal activity of coriander oil and its nanoemulsion. The characterization of nanoemulsion was also done.

Materials and Methods:

Essential oil: Coriander oil was obtained by steam distillation of coarsely grounded coriander seeds (*Coriandrum sativum*). Hundred grams of these ground seeds was hydro distilled in 500 ml distilled water for 90 min using steam distiller. The oil was then extracted using equal amount of diethylether (LobaChemie, Mumbai).

Organisms and culture conditions: The Amphotericin and Flucanazole resistant isolates of *C. albicans, C. tropicalis* and *C. parapsilosis* used in this study were obtained by sub culturing clinical specimens from the Microbiology department at the Breach Candy Hospital, Mumbai. The strains were maintained on Sabouraud's Dextrose medium prior to the experiments in presence of 20 microgram/ml Flucanazole (Forcan, Cipla). Cultures were propagated for two days in Sabouraud's medium at 37°C on an orbital shaker at 200 rpm. Cells were harvested by centrifugation, suspended in sterile saline and the optical density of cells adjusted to a final OD_{600} value of 1.0 using a colorimeter [9].

Nanoemulsion preparation: Nanoemulsion formulation was prepared using coriander oil, non-ionic surfactant Tween 80 (LobaChemie, Mumbai), cosurfactant Ethanol, Glycerol (LobaChemie, Mumbai) and water. Coarse emulsion was prepared by mixing 0.4 ml coriander oil in 1 ml each of Tween 80 and ethanol which was then heated to 80°C. To this, 2.4 ml water was added drop by drop followed by addition of 0.2 ml glycerol. This was then subjected to sonication for 15 min using a Sonicator (WENSAR, WUC-2L, ST-1011) at a high frequency of 40 kHz and power output of 50 W. The formulated nanoemulsion was then characterized.

Characterization of the nanoemulsion:

Droplet size and size distribution: The mean droplet size and polydispersity index of the nanoemulsion was determined by dynamic light scattering (DLS) (Delsa Nano, Beckman Coulter). Measurements were performed at 25°C using a scattering angle of 90°. The count rate was maintained to 1259 kcps.

Stability studies: The physical stability of a nanoemulsion formulation depends upon its preparation and mixing ratios of oil phase and aqueous phase. Thermodynamic stability tests were performed to overcome metastable formulation[10].

1. Centrifugation-The formulation was centrifuged at 3500rpm for 30 minutes [11].

2. Heating and cooling cycle - Six cycles between refrigerator temperature 4°C and 45°C with storage at each temperature of not less than 48 hours were done[11].

3. Freeze-thaw cycle - Three freeze-thaw cycles were done between -4°C and 25°C.

4. Zeta Potential Analysis - Dynamic light scattering was used to measure the zeta potential of the nanoemulsion by using Delsa Nano C, (Delsa Nano, Beckman Coulter). The measurement was carried out at a scattering angle of 90° at 25°C. In general, all the formulations that have high negative value of zeta potential charge above -30 mV indicates the stability of nanoemulsion formulations [13].

Antimicrobial assays:

Determination of MIC of coriander oil by broth dilution method (Tube assay): Minimum Inhibitory Concentration (MIC) of essential oils was determined by broth dilution method. A range of essential oil concentrations (2-30%) was prepared in Sabourauds Dextrose

broth (SDB) medium. To enhance essential oil solubility, Tween-80 was included at a final concentration of 0.5% (v/v) [13]. Each tube was inoculated with culture of OD_{600} of 1.0 to a final concentration of 0.1%. Tubes containing only Tween 80 (without plant essential oil) was kept as control. The tubes were then incubated for 48 hrs at 37°C. 0.1 ml of culture was taken from each tube (where growth was not observed) and inoculated on SDA plates and incubated at 37°C for 48 h. The plates were observed and MFCs (Minimum Fungicidal Concentration) were determined.

Determination of MIC of coriander oil nanoemulsion by broth dilution method (Tube assay)

Minimum Inhibitory Concentration (MIC) of essential oils was determined by broth dilution method. A range of nanoemulsion concentrations (2-20%) was prepared in Sabourauds Dextrose broth (SDB) medium. Each tube was inoculated with culture of OD_{600} of 1.0 to a final concentration of 0.1%. Tubes containing only Tween 80, ethanol, Glycerol as well as a combination of all three (without plant essential oil) was kept as control. The tubes were then incubated for a week at 37°C. 0.1 ml of culture was taken from each tube (where growth was not observed) and inoculated on SDA plates and incubated at 37°C for 48 h. The plates were observed and MFCs were determined.

Results and Discussions:

Droplet size: Coriander oil nanoemulsion was formulated with Tween 80, Ethanol and Glycerol. The average particle size was found to be 146.9 ± 1.9 nm and the typical size distributions of such dispersion are shown in Figure. 1. with a polydispersity index of 0.931, which reflects their relative homogeneity.

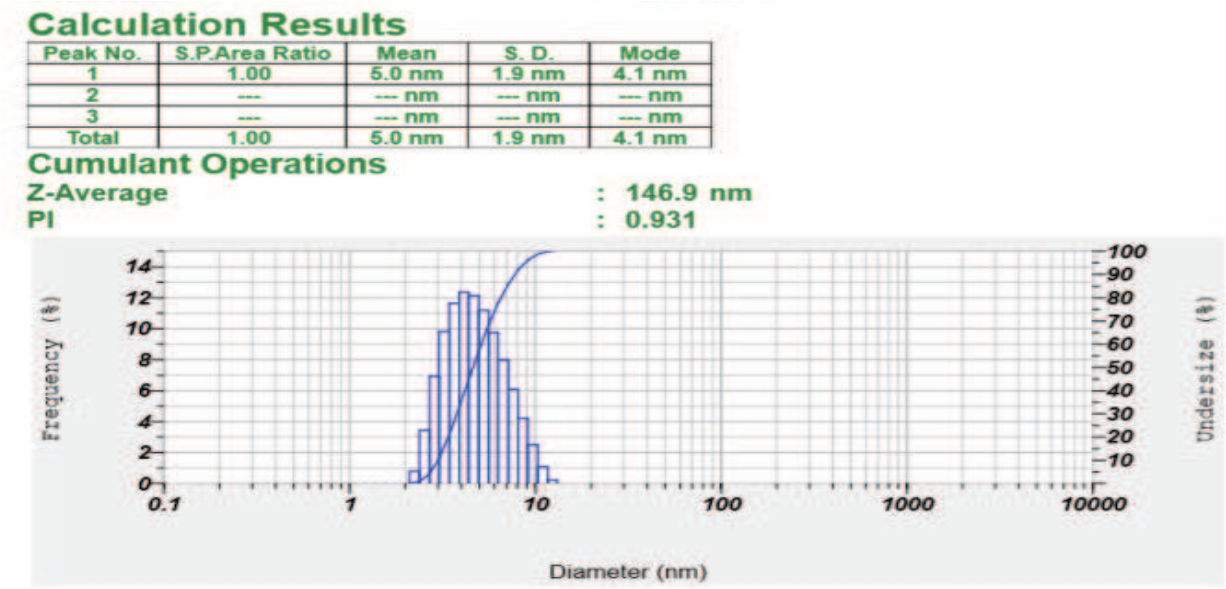

Figure1 represent the average diameter of the coriander oil nanoemulsion (146.9 nm) with a standard deviation of 1.9 nm and also gives its polydispersity index (PI)

Thermodynamic stability studies: Stress test including centrifugation, heating cooling cycle and freeze thaw cycles showed that the formulation had a good physical stability without any phase separation, creaming and flocculation. The zeta potential value was found to be-27.7mV represented by Figure. 2. The nanoemulsion is said to be stable if it has zeta

potential value approaching -30mV or lower. The negative zeta potential values presented by the samples are related to the presence of polysorbate 80, presenting a negative surface density of charge due to the presence of oxygen atoms in the molecules [14].Thus, it can be concluded that the nanoemulsion formulation was physically and chemically stable.

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-27.7 mV	-0.000214 cm2/Vs
2	— mV	— cm2/Vs
3	— mV	— cm2/Vs

Zeta Potential (Mean) : -27.7 mV

Electrophoretic Mobility mean : -0.000214 cm^2/Vs

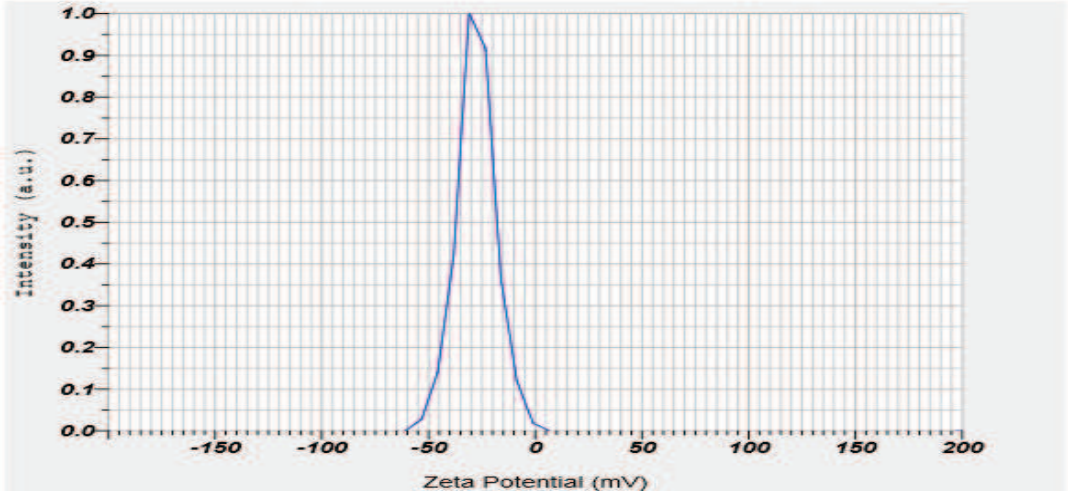

Figure 2 represent the zeta potential graph of the prepared coriander oil nanoemulsion formulation

Antimicrobial assay: The fungistatic and fungicidal concentrations of essential oil were determined by broth dilution method against *C. albicans, C. tropicalis* and *C. parapsilosis*. A comparison was carried out between the fungistatic and fungicidal activity of coriander oil and coriander oil nanoemulsion against all the three species of *Candida*. The coriander oil exhibited concentration-dependent inhibition of growth. A 20% concentration of coriander oil inhibited the growth of organism in all the three cases. Minimum fungicidal concentration (MFC) is defined as the lowest concentration of essential oil resulting in the death of 99.9% of the inoculum [15]. MFC was also found to be the same for all the three cultures and was observed to be same as MIC (20%).

However, MIC with respect to coriander nanoemulsion showed marked disparity as compared to coriander oil alone. MIC was found to be as low as 4% nanoemulsion concentration, signifying much lesser coriander oil

concentration (0.32%) with respect to all the three *Candida* spp. In general, it has been observed that the MFC was higher than MIC. It was found to be 8 % nanoemulsion concentration (0.64% coriander oil in nanoemulsion) for *C. parapsilosis* and 4 % nanoemulsion concentration for *C. tropicalis* and *C. albicans* (0.32% coriander oil in nanoemulsion). Also, the growth was seen after a week of incubation at 37°C indicating the probable long lag phase shown by the organisms.

Conclusion: Coriander oil nanoemulsion was prepared using ultrasonication method with a particle size of 146.9 ± 1.9 nm with fair stability. The nanoemulsion was found to be more potent against all the three *Candida* spp as compared to coriander oil alone. This is probably because the nanoemulsion particles are thermodynamically driven to fuse with lipid-containing organisms. This fusion is enhanced by the electrostatic attraction between the cationic charge of the emulsion and the anionic charge on the pathogen. When enough nanoparticles fuse with the pathogens, they release part of the energy trapped within the emulsion. Both the active ingredient and the energy released destabilize the pathogen lipid membrane, resulting in cell lysis and death.

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